SOFT LITHOGRAPHY FOR OLIGONUCLEOTIDE ARRAYS FABRICATION*

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Abstract: **A method for fabricating high-density oligonucleotide arrays directed by a set of molecular stamp was reported in this paper. In this method, based on the standard phosphoramidites chemistry protocol, the coupling was conducted under soft lithography. A 20-mer DNA microarray with 10 000 probes has been successfully fabricated, which shows the advantages of accurate, reliable operation and low-cost production of high-density DNA chip.**

Keywords-**oligonucleotide arrays, molecular stamping method, soft lithography, PDMS stamps, stamping coupling**

I. INTRODUCTION

With the complete DNA sequence of the human genome being determined, we are entering an era of genetic information. The high efficient and rapid method for detecting individual genome is attracting more and more attentions. Oligonucleotide arrays have been proving to be powerful tools for monitoring gene expression, resequencing genes to screen for mutations and polymorphism [1-3]. There are two main methods for DNA probe fabrication, which are sequential individual probe fixation and on-chip synthesis. In the former case, the probes are synthesized, purified and fixed in precise location in a sequential process. And this method is suitable for chips with low-density array. In the latter case, the four nucleotides are deposited successively on precise location. This technique is well suited for high-density chip fabrication. Several on-chip synthesis methods of oligonucleotide arrays have been reported in the past ten years [4-7]. For example, Fodor et al developed light-directed synthesis for the construction of high-density DNA probe arrays by using photolithography and solid-phase DNA synthesis [8]. Affymetrix Corporation has achieved DNA probe arrays with high spatial resolution. Our group in Southeast University has proposed an on-chip synthesis technology to fabricate the oligonucleotide arrays based on the molecular stamping and conventional DNA synthesis method [1,9]. Molecular stamp technology can also called as soft lithography initially developed by Whiteside group [10], which can achieve sub-microstructure (the spatial resolution of features of a PDMS stamp could reach up to 0.2 x 0.2 μm^2). Main advantages for soft lithography were simple and reliable operation. Once the stamp is available, multiple copies of the pattern can be produced using straightforward experimental techniques. In this paper, the principle of molecular stamping method was described and the preliminary result was reported.

II. THE MOLECULAR STAMPING METHOD

The basic strategy for the molecular stamping oligonucleotide synthesis accords to the standard phosphoramidites chemistry protocol and soft lithography, illustrates in Fig.1 and Fig.2. The surface of the glass substrate is treated so that it could bind single nucleotides (A、T、C、G). Stamping coupling is conducted as following. The mixed acetonitrile solution with nucleoside monomer and tetrazole as reactants is spread on features of the modification stamp, then transferred onto the modified substrate surface by machine alignment stamping until acetonitrile is vaporized to nearly dryness, therefore the nucleoside monomer on features of the stamp is coupled with the predefined regions on the substrate (Fig.1). According to the above-described details, a first stamp will cover most of the glass surface of the chip except for all

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Abstract

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sites which are destined to contain an oligonucleotide which begin with A, and these available sites are then coupled to adenosine; the next stamp allows other sites to couple T; a third allows the coupling of C, and a final one, the coupling of G. Next, oxidation, capping, and detritylation are conducted successively in a sealed reactor after four stamping couplings completed. Thus, through changing different stamps and their corresponding monomers the attachment of the first layer nucleotides is completed on their corresponding predefined regions, respectively. Nucleotide of the second layer is then added to each site with four other stamps. Accordingly, in order to synthesize N mer nucleotide on the chip Nx4 stamps were required and each stamp shows specific features. Fig. 2 was schematic illustration of the fabrication of the complete array with two nucleotides.

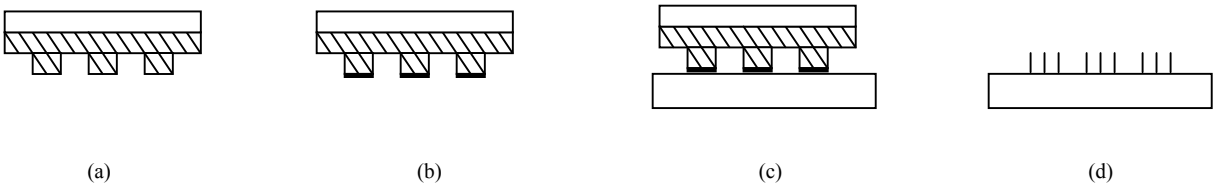


Fig.1. Schematic illustration of a stamping coupling. (a) stamp adhered to the glass plate, (b) spreading chemical reagents on the surface of the stamp, (c) delivering reactants onto the modification glass slide by stamping and then conducted coupling, (d) a new monomer was bond on the substrate

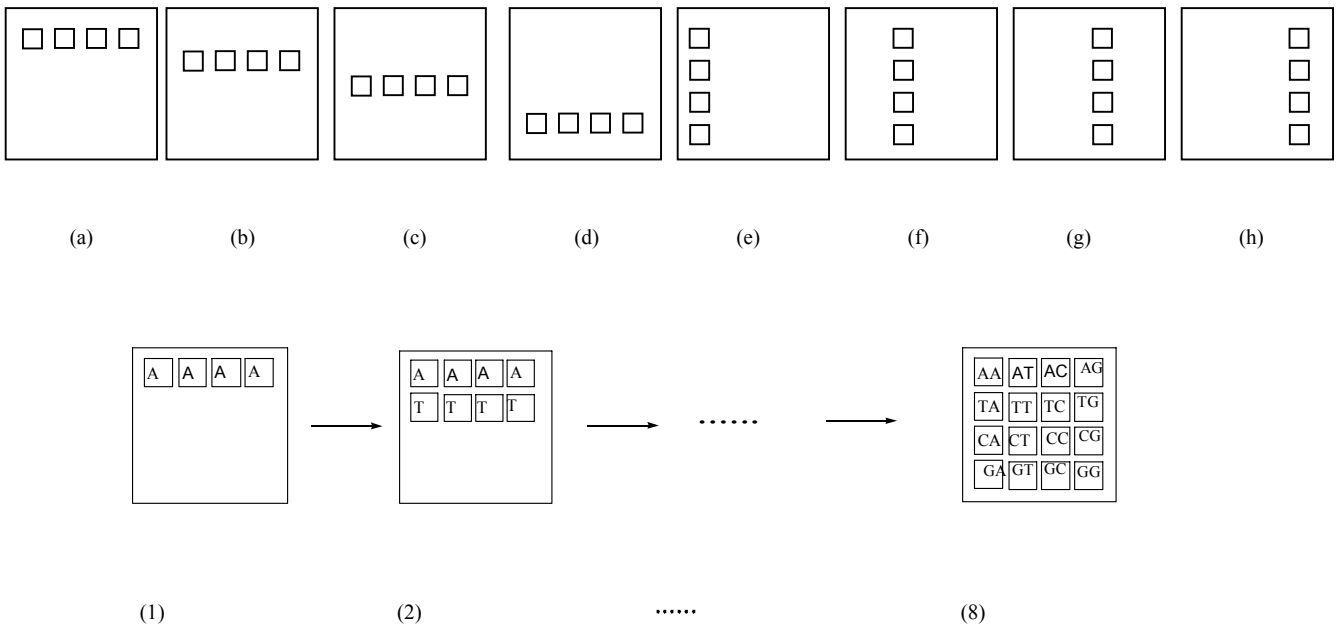


Fig.2. Schematic illustration of the fabrication of the complete array with two nucleotides through soft lithography and their corresponding 8 different stamps. （a）（b）（c）（d）four stamps comprised a set for synthesizing the first layer corresponding A, T, C, G four monomers; (d)（e）（f）（g）four stamps comprised another set for synthesizing the second layer corresponding A, T, C, G four monomers. (1), (2), ……, (8) express 8 different stamping coupling for synthesizing the complete array with two nucleotides.

III. MATERIALS AND METHODS

5'-DMT-2'-deoxynucleoside phosphoramidites (thymidine, N^4-isobutyryl-2'deoxycytosine,N^2-isobutyryl-2'-deoxy-guanosine,N^6-phenoxyacetyl-2'-deoxy-adenosine; Abbreviated T, C, G, A respectively), the other synthesis reagents and solvents except oxidation agent (seen in Table 1) were purchased from PE Biosystems. The glass substrates used for the coupling reaction were standard 'precleaned' soda limemicroscope slides purchased from the local stores. The commercially available polydimethlsiloxane was obtained from Hanzhou silicone rubber plant. Other chemical reagents were analytical grade and purchased from the local stores.

The modification of the glass slide [11]: A common sodium silicate glass slide was treated in H_2SO_4-$K_2Cr_2O_7$ solution for 24 h, and strongly washed with water, distilled water, then immersed in 5% aminopropyltrimethoxysilane in CH_3Cl for 5 min, washed successively with ether, acetone, and anhydrous ethanol, dried at 110℃ for 30 min. The slide was treated with 5% glutaradehyde in phosphate buffered saline (pH=7.4) for 2h, 10% aminoethyl alcohol for 2h, $NaBH_4$ solution for 15 min at ambient temperature respectively. The modified glass slide was dried at 110℃ for 30 min for oligonucleotides synthesis.

Preparation of the PDMS stamps [12]: Motherboards of stamps were made by lithography as described everywhere. By casting a mixed precursor including catalyst, ethylene silicate, and polydimethylsiloxane onto the motherboard whose surface had been lithographed, removing the bubbles in the precursor, and then covering the silanized glass slide on the mixed precursor; the sandwiched precursor was left to cure. After that, the motherboard was peeled off from the elastic cured polymer, so the stamp was fabricated. Then it was plasma-treated until hydrophilic surface formed, the stamp could be further used for oligonucleotide synthesis.In our experiment, each feature was $9.0x10^{-5}$m rectangle, i.e. 1 cm^2–sized arrays had 10 000 features.

Oligonucleotides synthesis: The synthesis was conducted in a glove box (Mecaplex, Switzerland) as detailed in and the concentration (by volume) of H_2O and O_2 in the glove box were about $2x10^{-4}$ %, $5x10^{-3}$ % respectively.

TABLE 1. CONDITIONS OF OLIGONUCLEOTIDE SYNTHESIS

step	reagents or solvent	time (second)
washing	acetonitrile	50
coupling*	0.1M phosphoramidite +0.5M tetrazole in acetonitrile	4x120
washing	acetonitrile	30
capping	Ac_2O/ Pyridine/N-methylimidazole in THF	30
washing	acetonitrile	30
oxidation	0.1M I_2/ Ac_2O/ AcOH/ Pyridine/THF	30
washing	acetonitrile	100
detritylation	3%TCA in CH_2Cl_2	50

*Coupling comprised 4 different stamping coupling

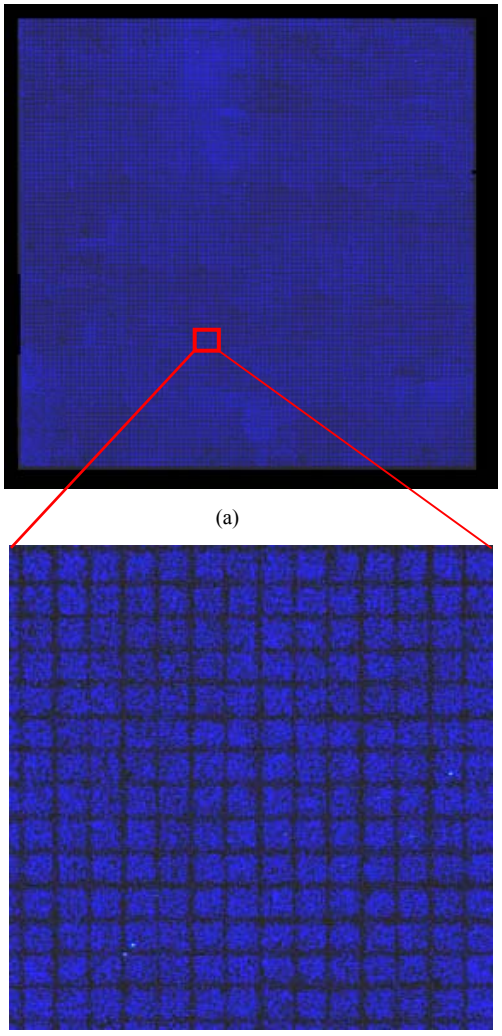
Oligonucleotide sequence was 3'-AGG AGG CTA AGT CTC TCA GG.

The deprotection, hybridization and detection of synthetic oligonucleotide arrays: After the synthesis, the glass slide was treated in a mixed solution of ethanol and aminoethyl alcohol (vol/vol=1) in a sealed box at 75℃ 2h for deprotection. Then it was washed with distilled water and dried by cold blowing before hybridization. Oligonucleotide arrays were hybridized in 200 nM 3'-TCC TCC GAT TCA GAG AGT CC- HEX (PE Biosystems) probe solution at 55℃ for 1.5h in the hybridization chamber, and then rinsed with 0.1% sodium dodecyl sulfate in 6xSSC (sodium chloride/sodium citrate buffer), 0.1% SDS in 0.1xSSC respectively. The probe array was scanned on the Scanarray Microarray Systems (Packard Biochip Technologies, USA). Once the scan was completed, a grid was aligned on the scanned image and a digitized intensity table was generated for each of the probe features on the chip.

IV. RESULTS

In this paper, the same oligonucleotide sequence was synthesized on the different features of the glass slide in order to confirm this soft lithography method feasible. Oligonucleotide arrays with the sequence 3'-AGG AGG CTA AGT CTC TCA GG was synthesized by soft lithography and the fluorescence images were shown in Fig. 6(a). Feature size was $9.0x10^{-5}$m rectangle and 1 cm^2–sized chip had 10 000 features. The fluorescence signal of all features on chip was well proportioned. The fluorescence intensities of synthetic and non-synthetic regions were 6478

and 582. This result was accordant to that of DNA automatic synthesizer and indicated this method was feasible. It was shown that the fluorescence intensity of oligonucleotide arrays rehybraized was approximate equal to that of the fresh chip even if the chip was in boiling water for tens hours or rehybraized many times. This meant oligonucleotide arrays synthesized on the glass substrate was steady and could be reused.



(a)

(b)

Fig.3. Hybridization to a matrix of 10k oligonucleotides. (a) Fluorescence image of 10K oligonucleotide array following hybridization with 3'-TCC TCC GAT TCA GAG AGT CC-HEX probe solution at 55℃; (b) the part of figure (a).

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